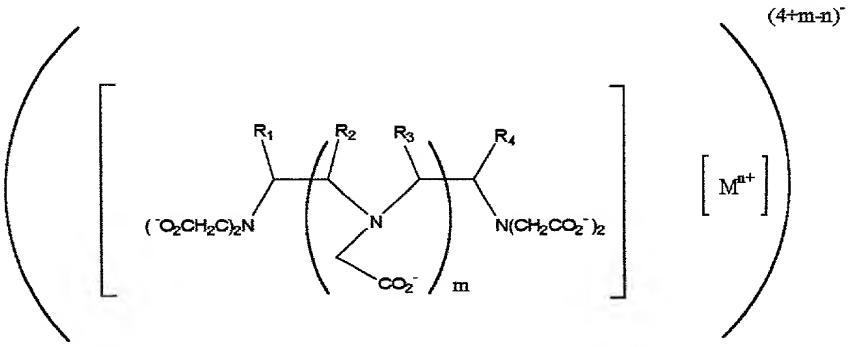
I claim:

1. A chelate-fluorophore tracer composition comprising:

a metal-chelated reagent having the formula

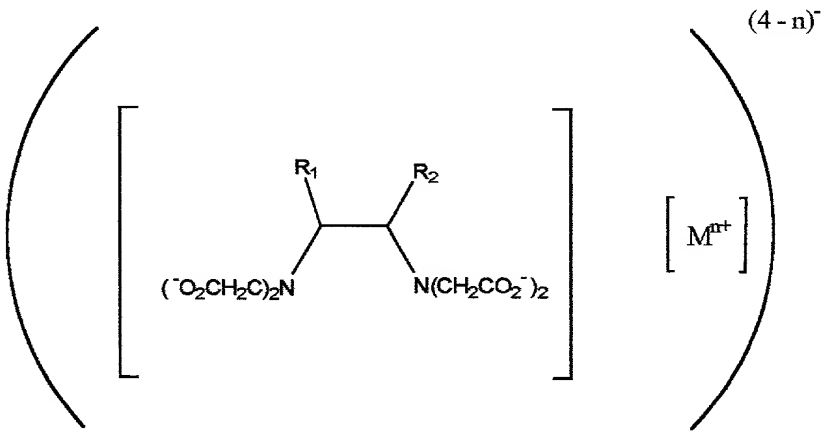


wherein m is 0 or 1; n is 1, 2, or 3; R_1 is $\underline{p}$-$CH_2C_6H_4$-X-Y or H, R_2 is H or $\underline{p}$-$CH_2C_6H_4$-X-Y, and R_3 and R_4 are H, CH_3, or are fused into a ring system; X is -HNC(S)NH-, -NHC(O)- or -NH-C_3N_3Cl-NH-; Y is a fluorophore having a fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization; and M is a metal chelated thereto selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, elements of Groups IIa, IIIa, IVa, Va, VIa, VIIa, VIII Ia, and VIII Ib of the Periodic Table of the Elements, elements of the lanthanide series of the Periodic Table of the Elements, and

elements of the actinide series of the Periodic Table of the Elements, excluding lawrencium.

2. A chelate-fluorophore tracer composition comprising:

a metal-chelated reagent having the formula

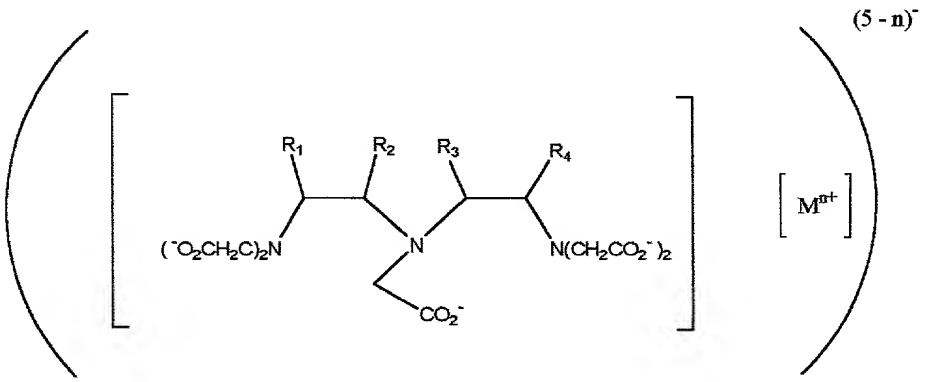


wherein n is 1, 2, or 3; R_1 is *p*-$CH_2C_6H_4$-X-Y, R_2 is H; X is -HNC(S)NH-, -NHC(O)- or -NH-C_3N_3Cl-NH-; Y is a fluorophore having a fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization; and M is a metal chelated thereto selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, elements of Groups IIa, IIIa, IVa, Va, VIa, VIIa, VIII Ia, and VIII Ib of the Periodic Table of the Elements, elements of the lanthanide series of the Periodic Table of the Elements, and

elements of the actinide series of the Periodic Table of the Elements, excluding lawrencium.

3. A chelate-fluorophore tracer composition comprising:

a metal-chelated reagent having the formula



wherein n is 1, 2, or 3; R_1 is *p*-$CH_2C_6H_4$-X-Y or H, R_2 is H or *p*-$CH_2C_6H_4$-X-Y, and R_3 and R_4 are H, CH_3, or are fused into a ring system; X is -HNC(S)NH-, -NHC(O)- or -NH-C_3N_3Cl-NH-; Y is a fluorophore having a fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization; and M is a metal chelated thereto selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, elements of Groups IIa, IIIa, IVa, Va, VIa, VIIa, VIII Ia, and VIII Ib of the Periodic Table of the Elements, elements of the lanthanide series of the Periodic Table of the Elements, and

elements of the actinide series of the Periodic Table of the Elements, excluding lawrencium.

4. The chelate-fluorophore tracer composition of Claim 2 or 3, further comprising the metal-chelated reagent wherein Y is selected from the group consisting of fluorescein derivatives, Texas red derivatives, rhodamine derivatives, coumarin derivatives, pyrene derivatives, naphthalene derivatives, and BODIPY dyes.

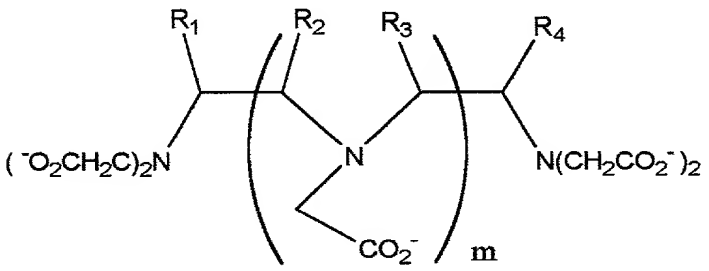
5. The chelate-fluorophore tracer composition of Claim 2 or 3, further comprising the metal-chelated reagent wherein M is a metal chelated thereto selected from the group consisting of bismuth, tin, lead, aluminum,gallium, indium, thallium, and elements of Groups IIa, IIIa, IVa, Va, Via, VIIa, VIII Ia, and VIII Ib of the Periodic Table of the Elements.

6. The chelate-fluorophore tracer composition of Claim 2 or 3, further comprising the metal-chelated reagent wherein M is a metal chelated thereto selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, cadmium, mercury, chromium, silver, antimony, barium, beryllium, thorium, zirconium, vanadium, nickel, molybdenum, manganese, zinc,

cobalt, iron, and copper.

7. A method for preparing a chelate-fluorophore tracer composition comprising:

a) adding a solution of a metal ion selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, elements of Groups IIa, IIIa, IVa, Va, VIa, VIIa, VIII Ia, and VIII Ib of the Periodic Table of the Elements, elements of the lanthanide series of the Periodic Table of the Elements, and elements of the actinide series of the Periodic Table of the Elements, excluding lawrencium, to an acidic solution of a fluorophore tracer composition comprising a chelating reagent having the formula



wherein m is 0 or 1, R_1 is *p*-$CH_2C_6H_4$-X-Y or H, R_2 is H or *p*-$CH_2C_6H_4$-X-Y, and R_3 and R_4 are H, CH_3, or are fused into a ring system; X is -HNC(S)NH-, -NHC(O)- or -NH-C_3N_3Cl-NH-; and Y is a fluorophore having a

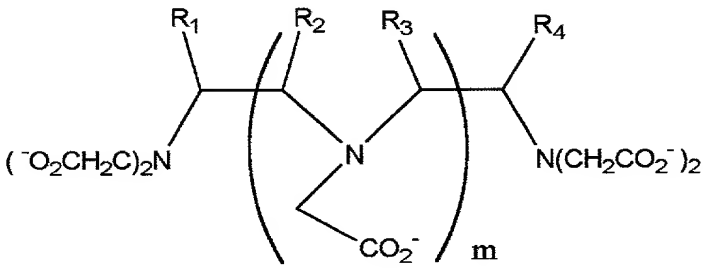
fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization; and

b) adjusting the pH of the resulting solution to about 7 or greater.

8. A method for preparing a chelate-fluorophore tracer composition comprising:

a) adding a solution of a metal ion selected from the group consisting of bismuth, tin, lead, aluminum, gallium, indium, thallium, elements of Groups IIa, IIIa, IVa, Va, VIa, VIIa, VIII Ia, and VIII Ib of the Periodic Table of the Elements, elements of the lanthanide series of the Periodic Table of the Elements, and elements of the actinide series of the Periodic Table of the Elements, excluding lawrencium, wherein the concentration of said metal ion in the solution is in the range from about 1 mM to about 20 mM, to a second, acidic solution of a fluorophore tracer composition

comprising a chelating reagent having the formula



wherein m is 0 or 1, R_1 is *p*-$CH_2C_6H_4$-X-Y or H, R_2 is H or *p*-$CH_2C_6H_4$-X-Y, and R_3 and R_4 are H, CH_3, or are fused into a ring system; X is -HNC(S)NH-, -NHC(O)- or -NH-C_3N_3Cl-NH-; and Y is a fluorophore having a fluorescence lifetime and quantum yield suitable for monitoring hapten-antibody binding at nanomolar concentrations by fluorescence polarization wherein the concentration of said fluorophore tracer composition is in the range from about 1 mM to about 20 mM, the metal to tracer composition stoichiometry is about 1.0-1.1:1.0, and the pH of said acidic solution is about 2 or lower; and

b) adjusting the pH of the resulting solution to about 7 or greater.

9. A method for evaluating the metal selectivity of a macromolecular biological binding agent comprising:

A) combining serial dilutions of an aqueous solution

thought to contain said biological binding agent with a fixed concentration of a first, target chelate-fluorophore tracer composition of Claim 1, wherein M is the target metal, and measuring the polarization of the fluorescent signal obtained when each resulting solution is excited with plane-polarized light;

B) combining identical dilutions of said aqueous solution thought to contain the biological binding agent with a second, non-target chelate-fluorophore tracer composition of Claim 1, wherein M is a non-target metal, said second tracer composition being present at the same concentration as the first tracer composition, and measuring the polarization of the fluorescent signal obtained when each resulting solution is excited with plane-polarized light;

C) subtracting the polarization signal produced by the solution containing the non-target tracer composition from that produced by the target tracer composition when measured at each sample dilution, whereby a positive net value at any dilution less than that producing a baseline signal for the target tracer composition indicates the presence of a macromolecular biological binding agent that binds

selectively to the target chelate-fluorophore composition and a zero or negative net value indicates no selectivity for said target chelate-fluorophore composition; and

D) repeating steps B) and C) for as many non-target metals as may be required to fully define the metal selectivity of the macromolecular binding agent according to its intended purpose.

10. A method for evaluating the metal selectivity of a polyclonal antibody response in a target chelate-immunized animal comprising:

A) combining serial dilutions of serum drawn from said animal with a fixed concentration of a first, target chelate-fluorophore tracer composition of Claim 1, wherein M is the target metal, and measuring the polarization of the fluorescent signal obtained when each resulting solution is excited with plane polarized light;

B) combining identical dilutions of said serum with a second, chelate-fluorophore tracer composition of Claim 1, wherein M is a non-target metal, said second tracer composition being present at the same concentration as the first tracer composition, and measuring the polarization of the fluorescent signal

obtained when each resulting solution is excited with plane polarized light;

C) subtracting the polarization signal produced by the solution containing the non-target tracer composition from that produced by the target tracer composition when measured at each sample dilution, whereby a positive net value at any dilution less than that producing a baseline signal for the target tracer composition indicates the presence of a polyclonal antibody that binds selectively to the target chelate-fluorophore composition; and

D) repeating steps B) and C) for as many non-target metals as may be required to fully define the metal selectivity of the polyclonal antibody according to its intended purpose.

11. A method for evaluating the metal selectivity of a monoclonal antibody present in a hybridoma supernatant or in a purified antibody preparation comprising:

A) combining serial dilutions of said hybridoma supernatant or purified antibody preparation with a fixed concentration of a first, target chelate-fluorophore tracer composition of Claim 1, wherein M is the target metal, and measuring the polarization of the fluorescent signal obtained when

each resulting solution is excited with plane polarized light;

B) combining identical dilutions of said hybridoma supernatant or purified antibody preparation with a second, chelate-fluorophore tracer composition of Claim 1, wherein M is a non-target metal, said second tracer composition being present at the same concentration as the first tracer composition, and measuring the polarization of the fluorescent signal obtained when each resulting solution is excited with plane polarized light;

C) subtracting the polarization signal produced by the solution containing the non-target tracer composition from that produced by the target tracer composition when measured at each sample dilution, whereby a positive net value at any dilution less than that producing a baseline signal for the target tracer composition indicates the presence of a monoclonal antibody that binds selectively to the target chelate-fluorophore composition; and

D) repeating steps B) and C) for as many non-target metals as may be required to fully define the metal selectivity of the monoclonal antibody according to its intended purpose.

12. An immunoassay method for determining the concentration of a target metal ion in an aqueous solution comprising:

A) combining an aliquot of said solution with a first assay reagent comprising a buffered solution of EDTA, DTPA, or a derivative thereof;

B) adding to the resulting solution a second assay reagent comprising the corresponding target chelate-fluorophore tracer composition of Claim 1, wherein M is the target metal;

C) adding to the second resulting solution a third assay reagent comprising a macromolecular biological binding agent that binds specifically to said target chelate-fluorophore tracer composition;

D) measuring the polarization of the fluorescent signal obtained when the third resulting solution is excited with plane-polarized light; and

E) comparing this value to those produced by standard solutions containing known concentrations of said target metal.

13. An immunoassay method for determining the concentration of a target metal ion in an aqueous solution comprising:

A) combining an aliquot of said solution with a

first assay reagent comprising a buffered solution of EDTA, DTPA, or a derivative thereof and the corresponding target chelate-fluorophore tracer composition of Claim 1, wherein M is the target metal;

B) adding to the resulting solution a second assay reagent comprising a macromolecular biological binding agent that binds specifically to said target chelate-fluorophore tracer composition;

C) measuring the polarization of the fluorescent signal obtained when the second resulting solution is excited with plane-polarized light; and

D) comparing this polarization value to those produced by standard solutions containing known concentrations of said target metal.

14. The immunoassay method of Claim 12 or 13, wherein the aqueous solution is obtained by extraction of a solid sample, or a multiphasic sample that contains solids, with one or more aqueous mineral acids.

15. The immunoassay method of Claim 12 or 13, wherein the aqueous solution is a water sample.

16. An immunoassay method for determining the

concentration of lead in an aqueous extract of a solid sample, or of a multiphasic sample that contains solids, comprising:

A) combining an aliquot of said aqueous extract with a first assay reagent comprising a buffered solution of EDTA or a derivative thereof and the corresponding target chelate-fluorophore tracer composition of Claim 3, wherein M is lead;

B) adding to the resulting solution a second assay reagent comprising a biological binding agent that binds specifically to said target chelate-fluorophore tracer composition;

C) measuring the polarization of the fluorescent signal obtained when the second resulting solution is excited with plane-polarized light; and

D) comparing this polarization value to those produced by standard solutions containing known concentrations of lead(II).

17. An immunoassay method for determining the concentration of lead in a water sample, comprising:

A) combining an aliquot of said water sample with a first assay reagent comprising a buffered solution of EDTA or a derivative thereof and the corresponding target chelate-fluorophore tracer

composition of Claim 3 wherein M is lead;

B) adding to the resulting solution a second assay reagent comprising a biological binding agent that binds specifically to said target chelate-fluorophore tracer composition;

C) measuring the polarization of the fluorescent signal obtained when the second resulting solution is excited with plane-polarized light; and

D) comparing this polarization value to those produced by standard solutions containing known concentrations of lead(II).

18. The immunoassay method of Claim 16 or 17, further comprising an assay diluent comprising between about 10 - 100 mM sodium bicarbonate or HEPES, between about 10-100 μM EDTA, and between about 1- 10 nM lead chelate-fluorophore tracer composition of Claim 1 wherein M is Pb, n is 2, R_1 is *p*-$CH_2C_6H_4$-X-Y, R_2 is H; X is -HNC(S)NH-; and Y is fluorescein.

19. The immunoassay method of Claim 16 or 17, further comprising an antibody comprising the rabbit polyclonal antiserum raised against the EDTA-Pb complex and screened for cross-reactivity with aluminum, iron, chromium, zinc, copper, and nickel.

20. A test kit for measuring the concentration of a target metal in a test sample, comprising:

A) at least one standard solution containing a known concentration of the target metal;

B) a first assay reagent comprising a base, a chelating agent, and the corresponding target metal chelate-fluorophore tracer composition of Claim 1 wherein M is the target metal; and

C) a second assay reagent containing a known concentration of the biological binding agent responsive to the target metal chelate-fluorophore tracer composition.

21. A test kit for measuring the concentration of lead in a test sample, comprising:

A) at least one standard solution containing a known concentration of lead(II);

B) a first assay reagent comprising a base, EDTA, and the corresponding lead chelate-fluorophore tracer composition of Claim 3 wherein n is 1, Y is fluorescein, and M is Pb;

C) a second assay reagent containing a known concentration of the biological binding agent responsive to the lead chelate-fluorophore tracer composition; and

D) optionally, an extraction fluid suitable for obtaining an aqueous extract of a solid sample, or a multiphasic sample that contains solids.

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